Amendments to the Specification

Please replace the paragraph at page 1, lines 7-14 with the following paragraph:

This application is a continuation application of U.S. Serial No. 08/713,556, filed on August 30, 1996, ~~Pending,~~ now issued U.S. Patent No. 6,277,975, which in turn is a continuation-in-part of U.S. Serial No. 08/428,734, filed April 25, 1995, issued U.S. Patent No. 5,843,707, which was a continuation-in-part of copending application U.S. Serial No. 08/316,305, filed September 30, 1994, which was a continuation-in-part of copending application U.S. Serial No. 08/235,398, filed April 28, 1994, which was a continuation-in-part of copending application U.S. Serial No. 08/112,608, filed August 26, 1993, which was a continuation-in-part of U.S. Serial No. 07/965,662, filed October 23, 1992, now abandoned. This application also claims priority from International Application No. PCT/US93/10168, filed October 22, 1993. The contents of all of the aforementioned application(s) are hereby incorporated by reference.

Please replace paragraph at page 8, lines 26-27, with the following paragraph:

Fig. 22 depicts a proposed model for binding of P-selectin ligand proteins to P- and E-selectin. (See Example 10). The amino acid sequence for the PSGL-1 molecule is represented by QATEYEYLDYDFLPETEPPEMLRNSTDTTPT (SEQ ID NO:48).

Please replace paragraph at page 9, lines 31-32, with the following paragraph:

Fig. 30 summarizes the results of screening of various P-~~selecint~~ selectin ligand proteins for inhibition of P- and E-selectin binding (See Example 13). The peptides

used in the screening assay were: QATEYEYLDYDFLPEC (SEQ ID NO:43); TEYEYLDYDF (amino acid 3 to 12 of SEQ ID NO:43); SYLDYS (SEQ ID NO:44) without any modifications, with one tyrosine sulfonated, with one tyrosine phosphorylated, or with both tyrosines phosphorylated; SFLDYS (SEQ ID NO:45) with one tyrosine phosphorylated; and YLDY (amino acid 2 to 5 of SEQ ID NO:44) with both tyrosines phosphorylated.

Please replace the paragraph starting at page 9, line 34 and ending at page 10, line 16 with the following paragraph:

The present inventors have for the first time identified and isolated a novel DNA which encodes a protein which acts as a ligand for P-selectin on human endothelial cells and platelets. The sequence of the DNA is set forth in SEQ ID NO:1. The complete amino acid sequence of the P-selectin ligand protein (i.e., the mature peptide plus the leader sequence) is characterized by the amino acid sequence set forth in SEQ ID NO:2 from amino acid 1 to amino acid 402. Hydrophobicity analysis and comparison with known cleavage patterns predict a signal sequence of 20 to 22 amino acids, i.e., amino acids 1 to 20 or amino acids 1 to 22 of SEQ ID NO:2. The P-selectin ligand protein contains a PACE (paired basic amino acid converting enzyme) cleavage site (-Arg-Asp-Arg-Arg-) (SEQ ID NO:46) at amino acids 38-41 of SEQ ID NO:2. The mature P-selectin ligand protein of the present invention is characterized by the amino acid sequence set forth in SEQ ID NO:2 from amino acid 42 to amino acid 402. A soluble form of the P-selectin ligand protein is characterized by containing amino acids 21 to 310 of SEQ ID NO:2. Another soluble form of the mature P-selectin ligand protein

is characterized by the amino acid sequence set forth in SEQ ID NO:2 from amino acid 42 to amino acid 310. The soluble form of the P-selectin ligand protein is further characterized by being soluble in aqueous solution at room temperature. Of course, the corresponding DNA sequences as set forth in SEQ ID NO:1 encoding these proteins are also included in the subject invention.

Please replace the paragraph at page 11, lines 5-16, with the following paragraph:

The structure of the full-length P-selectin ligand protein is schematically represented in Fig. 5. Three regions of the P-selectin ligand protein of SEQ ID NO:2 are: an extracellular domain (from about amino acid 21 to 310 of SEQ ID NO:2), a transmembrane domain (from about amino acid 311 to 332 of SEQ ID NO:2), and an intracellular, cytoplasmic domain (from about amino acid 333 to 402 of SEQ ID NO:2). The extracellular domain contains three consensus tripeptide sites (Asn-X-Ser/Thr) of potential N-linked glycosylation beginning at Asn residues 65, 111, and 292. The extracellular domain further contains three potential sites of tyrosine sulfation at residues 46, 48, and 51. The region comprised of residues 55-267 contains a high percentage of proline, serine, and threonine including a subdomain of fifteen decameric repeats of the ten amino acid consensus sequence Ala-Thr/Met-Glu-Ala-Gln-Thr-Thr-X-Pro/Leu-Ala/Thr (SEQ ID NO:47), wherein X can be either Pro, Ala, Gln, Glu, or Arg. Regions such as these are characteristic of highly O-glycosylated proteins.

Please replace the bridging paragraph beginning at page 12, line 35 and ending at page 13, line 23, with the following paragraph, noting that the number 10 on page 13, line 20, and the number 114 on page 13, line 21, were underlined in the specification as originally filed, are not amendatory matter, and should continue to be underlined:

Fragments of the P-selectin ligand protein which are capable of interacting with P-selectin or which are capable of inhibiting P-selectin-mediated intercellular adhesion are also encompassed by the present invention. Such fragments comprise amino acids 21 to 54 of SEQ ID NO:2, a region of the P-selectin ligand protein having a low frequency of serine and threonine residues; amino acids 55 to 127 of SEQ ID NO:2, having a high frequency of proline, serine, and threonine in addition to two consensus sequences for asparagine-linked glycosylation (Asn-X-Ser/Thr); another larger fragment, amino acids 128 to 267 of SEQ ID NO:2, having both a high frequency of proline, serine, and threonine and containing fifteen repeats of the following ten amino acid consensus sequence: Ala-(Thr/Met)-Glu-Ala-Gln-Thr-Thr-(Pro/Arg/Gln/Ala/Glu)-(Leu/Pro)-(Ala/Thr) (SEQ ID NO:47)(smaller fragments within this large fragment may also retain the capacity to interact with P-selectin or act as inhibitors of P-selectin-mediated intercellular adhesion); the region containing a consensus sequence for asparagine-linked glycosylation and comprising amino acids 268 to 308 of SEQ ID NO:2; the hydrophobic region of the protein represented by amino acids 309 to 333 of SEQ ID NO:2; and the amphophilic region of the P-selectin ligand protein from amino acids 334 to 402 of SEQ ID NO:2. Additional fragments may comprise amino acid 43 to amino acid 56 of SEQ ID NO:2 or amino acid 42 to amino acid 60 of SEQ ID NO:2, with one or more sulfated or phosphorylated (Domcheck *et al.*, Biochemistry *31*:9865-9870

(1992)) tyrosines at amino acid 46, amino acid 48, and/or amino acid 51. Fragments of the P-selectin ligand protein may be in linear form or they may be cyclized using known methods, for example, as described in H.U. Saragovi, *et al.*, Bio/Technology 10, 773-778 (1992) and in R.S. McDowell, *et al.*, J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. For the purposes of the present invention, all references to "P-selectin ligand protein" herein include fragments capable of binding to P-selectin.

Please replace the paragraph at page 19, lines 27-36, with the following paragraph, noting that the reference to Texas Agricultural Experiment Station Bulletin No. 1555 (1987) was underlined in the specification as originally filed, is not amendatory matter, and should continue to be underlined:

The P-selectin ligand protein may also be produced by operably linking the isolated DNA of the invention and one or more DNAs encoding suitable glycosylating enzymes to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, California, U.S.A. (the ~~MaxBac~~MAXBAC® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. Soluble forms of the P-selectin ligand protein may also be produced in insect cells using appropriate isolated DNAs as described above. A DNA encoding a form of PACE may further be co-

expressed in an insect host cell to produce a PACE-cleaved form of the P-selectin ligand protein.

Please replace the bridging paragraph at page 20, line 33 to page 21, line 13 with the following paragraph:

The P-selectin ligand protein of the invention may be prepared by culturing transformed host cells under culture conditions necessary to express a P-selectin binding glycoprotein. The resulting expressed glycoprotein may then be purified from culture medium or cell extracts. Soluble forms of the P-selectin ligand protein of the invention can be purified by affinity chromatography over Lentil ~~lectin~~ lectin-SEPHAROSE® and subsequent elution with 0.5 M α-methyl-mannoside. The eluted soluble P-selectin ligand protein can then be further purified and concentrated by a 0-70% aluminium sulfate precipitation step. The protein is then recovered, resuspended, and further purified by size exclusion chromatography over a TSK G4000SW_{XL}. Alternatively, full-length forms of the P-selectin ligand protein of the invention can be purified by preparing a total membrane fraction from the expressing cell and extracting the membranes with a non-ionic detergent such as Triton X-100. The detergent extract can then be passed over an affinity column comprised of immobilized P-selectin, and the P-selectin ligand protein can be eluted from the column with 10mM EDTA in a buffer containing 0.1% detergent. The material eluted from the affinity column can then be dialyzed to remove EDTA and further purified over a Lentil ~~lectin-Sepharose~~ lectin-SEPHAROSE® affinity column, again eluting with 0.5M α-methyl-mannoside.

Please replace the paragraph at page 21, lines 14-26, with the following paragraph:

Alternatively, the P-selectin ligand protein of the invention is concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred (e.g., ~~S-Sepharose~~ S-SEPHAROSE® columns). The purification of the P-selectin ligand protein from culture supernatant may also include one or more column steps over such affinity resins as concanavalin A-agarose, ~~heparin-toyoperal~~ heparin-TOYOPEARL® or Cibacrom blue 3GA ~~Sepharose~~ SEPHAROSE®; or by hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or by immunoaffinity chromatography.

Please replace the paragraph at page 43, lines 31-36, with the following paragraph:

Autoradiography of the materials released from the resins by EDTA was performed by electrophoresis of samples (approximately 10,000 cpm samples concentrated by Centricon-10 units where needed) on 10% cross-linked SDS-PAGE

gels, subsequent treatment of the gels with EN3HANCE™ (~~Dupont~~DUPONT™) as per the manufacturer's instructions followed by drying for two hours on a commercially available gel dryer (Bio-Rad). Exposure of the dried gels to X-ray film was conducted for a minimum of three days at -80 degrees C.